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Integrated bioprocessing in Saccharomyces cerevisiae using green fluorescent protein as a fusion partner.

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In this study, we examine the use of green fluorescent protein (GFP) for monitoring a hexokinase (HXK)-GFP fusion protein in Saccharomyces cerevisiae for various events including expression, degradation, purification, and localization. The fusion, HXK-EK-GFP-6 x His, was constructed where the histidine tag (6 x His) would allow for convenient affinity purification, and the enterokinase (EK) cleavage site would be used for separation of HXK from GFP after affinity purification. Our results showed that both HXK and GFP remained active in the fusion and, more importantly, that there was a linear correlation between HXK activity and GFP fluorescence. Enterokinase cleavage studies revealed that both GFP fluorescence intensity and HXK activity remained unchanged after separation of the fusion proteins, which indicated that fusion of GFP did not cause structural alteration of HXK and thus did not affect the enzymatic activity of HXK. We also found that degradation of the fusion protein occurred, and that degradation was limited to HXK with GFP remaining intact in the fusion. Confocal microscopy studies showed that while GFP was distributed evenly in the yeast cytosol, HXK-GFP fusion followed the correct localization of HXK, which resulted in a di-localization of both cytosol and the nucleus. GFP proved to be a useful fusion partner that may lead to the possibility of integrating the bioprocesses by quantitatively following the entire process visually.